Proceedings of 8th International Conference on Mushroom Biology and Mushroom Products

Volume II

World Society of Mushroom Biology and Mushroom Products
ICAR-Directorate of Mushroom Research, Solan
Mushroom Society of India, Solan

MUSHROOM SCIENCE IN CUBA: TOWARDS NEW OPPORTUNITIES FOR DEVELOPING FUNCTIONAL FOODS/NUTRACEUTICALS

HUMBERTO J MORRIS*, ROSA C BERMÚDEZ, GABRIEL LLAURADÓ, YAIXA BELTRÁN AND NORA GARCÍA

Center for Studies on Industrial Biotechnology (CEBI), Universidad de Oriente, Reparto Jiménez, Santiago de Cuba 5. CP 90 500, Cuba
hmorris@cebi.uo.edu.cu

ABSTRACT

Mushroom science in Cuba allows the valorization of agricultural by-products into functional foods/nutraceuticals for human consumption to address objectives of sustainability and biotechnological development. Much research work done in Cuban eastern region has been performed on the *Pleurotus* genus, whose cultivation has increased greatly during the last few decades. *Pleurotus* species, like many edible and medicinal mushrooms, are a good source of immunomodulators and "host defense potentiators" (HDPs). In this context, dietetic supplements with a high therapeutic potential acting on the immune system and formulated from refined or partially refined mushroom extracts, or from dried mycelia/fruiting bodies biomass are referred as "mushroom immunoceuticals". The present study examined the synergy exerted by the structural diversity of biomolecules found in *Pleurotus* crude extracts and powders on immune responses of both immunocompetent and immunodeficient Balb/c mice. *Pleurotus* derived-products could potentiate the host defense mechanisms *in vivo* and should be promising for further pharmacological studies. The effects on cell immunity are especially valuable in the prophylaxis of tumors, immunodeficiencies and as co-adjuvant in chemotherapy. The results also demonstrate that not only mushrooms but also their mycelia may be a good candidate for nutraceuticals production. Through this immunological "window" we are assisting to a revolution in mushroom science characterized by the diversity of compounds found in mushrooms and on the other hand, by the possibilities given by the abundance of specific molecular targets. An extended knowledge of the immuno-enhancing activity of *Pleurotus* nutraceuticals would be useful in understanding their potential applications for immunonutrition and immunotherapy.

Keywords: *Pleurotus*, edible and medicinal mushrooms, functional foods, nutraceuticals, immunomodulating activity

INTRODUCTION

Today the well-being of humankind faces unprecedented challenges involving inadequate regional food supplies, deficiency in new insight into healthy eating, diminishing quality of health, and increasing environmental deterioration. Therefore, we live in an age of human health crises, especially when considering the leading killer diseases of our time such as cancer, HIV/AIDS and the upsurge of hypertension, diabetes, cardiovascular disorders and obesity worldwide. The magnitude of these problems is set to increase as the world's population continues to grow [1]. A cost analysis made in 2010 at Harvard´s University suggested that if present health tendencies are not reverted, the costs due to medical services associated to chronic non-transmissible diseases will rise to 47 USD trillions in the next 20 years [2]. Cuba is not an exception in this international scene and Table 1 shows the statistics for the main causes of mortality caused by these diseases in 2013 [3].

Taking into account that the main health determinants involve the environment, the life-styles, human biology and the medical assistance and that of these factors diet is the determinant to which all of us are daily exposed, the challenge now is to drastically change the food habits [4]. In this context, the use of mushrooms, past and present, and practices, represent an important cultural heritage as they have been used since immemorial times as food and medicine according to traditional ecological knowledge transmitted along generations; its current use is supported by scientific evidences [5, 6]. Mushrooms have long been valued as highly tasty/nutritional foods with an established history of use in traditional oriental therapies and modern clinical practice in several Asian countries [7, 8].

In the last five years, the consumption of mushrooms, either as whole mushroom or extracted supplements has increased [9]. Most mushroom-derived preparations (extracts, powders and tablets) are usually included in the following categories of products: dietary supplements, functional foods, nutraceuticals, nutriceuticals, phytochemicals and design foods [10, 11]

Table 1. Selection of the main causes of mortality for all groups of age in Cuba (2013)*

Diseases	Number of deaths	Mortality(number of deaths/ 100 000 inhabitants)
Cancer	22 868	204.8
Cardiovascular diseases	22 651	202.9
Cerebrovascular diseases	9 011	80.7
Influenza and pneumonia	6 091	54.6
Diabetes	2 246	20.1
VIH/SIDA	353	3.2

*Adapted from the Cuban Health Statistical Annals (2013).

and immunoceuticals [12]. Therefore, the significant impact of mushroom cultivation and mushroom products on long-term food nutrition, health enhancement and human welfare in the 21st century could be considered globally as a non-green revolution [13].

The verification of beneficial effects pointing mainly to the reduction of risk factors for chronic non-transmissible diseases is the major issue in the science of functional foods. Medicinal effects have been demonstrated for many traditionally used mushrooms, including extracts of species from genera *Auricularia, Flammulina, Ganoderma, Grifola, Hericium, Lentinus* (*Lentinula*), *Pleurotus, Trametes* (*Coriolus*), *Schizophyllum,* and *Tremella* [9, 14].

The number of recognized mushroom species has been reported to be 14,000 which account for 10% of the estimated 140 000 mushroom species. Of the recognized mushroom species, about 7,000 (50%) are considered to possess varying degrees of edibility and approximately 700 are considered to be safe species with medicinal properties [9, 15]. Therefore, mushrooms represent a major and yet largely untapped source of powerful new functional and pharmacological products [16].

Strictly speaking, mushroom biodiversity in Cuba is not estimated. The Cuban National Botanical Garden presented a list of 97species belonging to 59 genera, 31families and 11orders, standing out for their representativeness the families Polyporaceae and Coprinaceae. In the study, 17 species were identified as edible and six possessed medicinal properties [17]. Thus the evaluation of the genetic and phenotypic biodiversity and the effects of variations in environmental factors on the quality of products (edible mushroom and active ingredients) are needed.

Unlike Latin American countries, edible mushrooms are not part of the culinary tradition of the Cuban people. Until a few decades, *Pleurotus* was the only genus of mushroom cultivated in Cuba for human consumption. Its cultivation began in 1988 at the Cuban Research Institute for Sugar Cane Derivatives (ICIDCA, Havana) using sugar cane bagasse as substrate [18]. Taking into account that Cuban eastern mountainous region produces about 80% of the high quality coffee in the island, and as a result large amounts of organic by-products like coffee pulp are generated, the Center for Studies on Industrial Biotechnology (CEBI, Santiago de Cuba) began to use these lignocellulosic materials as substrates for *Pleurotus* cultivation [19].This is an efficient biotechnological process for their bioconversion and recycling as a sustainable model for rural production in agreement with the "cluster thought" of agriculture in the 21st century [1].Other activities in Cuban eastern region generate several by-products (cocoa shells, coconut husks) also used as substrates for mushrooms production both in CEBI experimental unit and in a rural mushroom farm [19].

Recently, the Ministry for Foreign Investment and Economic Cooperation of Cuba and the Food and Agriculture Organization of the United Nations (FAO) signed a Technical Cooperation Project devoted to increase the cultivation of edible fungi, mainly *Volvariella volvacea* using rice straw as a substrate.The main executor of the project is the Institute of Fundamental Research in Tropical Agriculture (INIFAT, Havana), and now is part of the urban agriculture program of that institution.

Why mushroom research in Cuba? The Cuban Social Policy guidelines for Health declare: *"the need to pay the highest attention to the development of natural and traditional medicine"* as well as *"to strengthening promotion and prevention actions that delay or prevent the onset of chronic non-transmissible diseases and their sequelae"*. In this context, the CEBI´s Biotechnology of Edible and Medicinal Mushrooms group is aimed *"To carry out innovative research/ development activities in Biotechnology of Edible and Medicinal Mushrooms aimed to obtain new natural products with potential applications as dietetic supplements and/or biopharmaceuticals, thus achieving a favorable impact on public health"*.

Why *Pleurotus* genus? This genus comprises some of the most popular *Basidiomycetes* edible mushrooms whose cultivation has increased greatly throughout the world during the last few decades [20]. Its popularity has been expanded due to its vigorous growth on a variety of agroforestry substrates and for the production of a high nutritional value-food [21] containing compounds with therapeutic effects [22]. On the other hand, recent studies on *Pleurotus* spp. have shown a number of pharmacological activities, such as antitumour, immunomodulatory, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antihyperglycaemic, antimicrobial and antiviral activities [23].

With the view of developing new therapeutic agents to potentiate host resistance to cancer and infectious disease, such as AIDS, there has been an upsurge of interest in immunomodulating substances from medicinal mushrooms [16, 24]. *Pleurotus* species, like many edible and medicinal mushrooms, are a good source of immunomodulators and substances considered as "host defense potentiators" (HDPs) as judged by their immunostimulating properties. Several molecules able to augment or complement a desired immune response have been isolated from *Pleurotus* spp., particularly polysaccharides. These compounds stimulate different cell populations of the immune system, for instance, macrophages, Natural Killer (NK) cells, T cells, and also modulate cytokine system [24, 25]. The study of the synergy exerted by the vast structural diversity of biomolecules found in *Pleurotus* crude extracts, powders and other preparations on immune responses deserves special attention [26].

In Cuba, the implementation of technologies for the cultivation of *Pleurotus* spp. on agricultural substrates, in addition to food generation for human consumption opened new research activities towards mushroom immunoceuticals. In addition to the use as functional foods, *Pleurotus* fruiting bodies and mycelia obtained under Good Manufacture Practices can be applied in the formulation of nutraceuticals and biologically active products.

Through different examples of recent research made in our laboratory, this paper illustrates the effects of *Pleurotus* sp. crude extracts and powders on immune responses of both immuno competent and immuno deficient Balb/c mice. An extended knowledge of the immuno-enhancing activity of *Pleurotus* functional foods/ nutraceuticals would be useful in understanding their potential applications for immunonutrition and immunotherapy.

MATERIALS AND METHODS

Mushroom material

Pleurotus sp. strain (CCEBI-3024) is deposited at the Culture Collection of the Center for Studies on Industrial Biotechnology (CEBI, Cuba). The strain was maintained on slants with solid medium of potato dextrose agar (PDA) incubated at 5 °C.

Preparation of *Pleurotus*-derived products

Pleurotus sp. cultivation was performed by solid-state fermentation of mushroom spawn on pasteurized coffee pulp used as substrate in plastic bags of 2 kg (30-40 cm) [19]. The fruiting bodies were harvested, sliced into small pieces and dried at 45 °C for 24 h. The dried material (*Pleurotus*-DP) was milled, and the resulting powder was preserved away from light and humidity in plastic bags for further use.

For obtaining the *Pleurotus* fruiting bodies cold water-extract (CW-E), the collected carpophores were exhaustively washed with distilled water and sliced into 1 cm^2 pieces. They were weighed and 5 ml of distilled water was added per gram of biological material. The extraction was made at 20 °Cwith continuous stirring at 100 rpm for three hours and the final extracts were collected by centrifugation and filtration. The extracts were stored at -20 °C and freeze dried. They are mainly composed of 43% of carbohydrate and 35% of protein.

The preparation of *Pleurotus* mycelium hot-water extract (HW-E) started with the inoculation of mycelium in Erlenmeyer flasks, which contained YPG medium (yeast-peptone-glucose). The flasks were incubated at 27 °C with continuous stirring at 100 rpm for 15 days. After the submerged fermentation was carried out, mycelia were collected by centrifugation at 4000 rpm and washed twice with distilled water. Isolated mycelia, suspended in 200 g (wet weight)/L of distilled water, were extracted with boiling water for 10 h and the final extracts were collected by centrifugation and filtration. The extracts were stored at -20 °C and freeze dried. The major components of HW-E were carbohydrate (76.8%) and protein (12%).

Mycochemical profile of *Pleurotus*-derived products

The fpowder of ruiting bodies was extracted with hot-water to obtain an aqueous extract for assessing its mycochemical profile. The metabolites contained in *Pleurotus*-derived products were estimated qualitatively [27].

Laboratory animals

Balb/c mice purchased from the National Center for Production of Laboratory Animals (CENPALAB, Havana) were used. Experiments were done under conventional sanitary conditions and animals were maintained at controlled temperature and humidity throughout the investigation ensuring the optimal interval for the specie. The administration of products was made daily in the morning between 9-10 am. The research was approved by the institutional Ethical Committee (University of Oriente) and has been performed in accordance with Cuban legislation and the National Research Council Guidelines for the Care and Use of Laboratory Animals.

Effect of oral administration of *Pleurotus* fruiting bodies powder to immune competent Balb/c mice

Both female and male Balb/c mice of eight weeks weighing between 18-25 g were used. Mice were fed a standard diet and acidified water *ad libitum*. Twenty mice were randomly divided into four groups (n=5), two groups belonging to each sex. The groups identified as *Pleurotus*-DP females and *Pleurotus*-DP were administered orally for 14 days with 0.2 ml of a freshly prepared suspension of fruiting bodies powder in saline solution, equivalent to a dose of 1000 mg/kg of body weight, as a supplement of the standard pelleted diet. Control-females and -males groups were fed with the conventional diet throughout the investigation. On day 15, blood samples were collected from the orbital vein of mice for total and differential leukocyte counts. For differential counts, a blood sample was placed onto glass slides, fixed with methanol, and then stained with Giemsa solution.

In a parallel-conducted experiment, the cell-mediated immune response was assessed by the delayed-type hypersensitivity reaction (DTH). Animals were immunized by an intradermal (i.d.) injection of 50 µl of 5 mg/ml bovine serum albumin (BSA) emulsified in Complete Freund Adjuvant (CFA) (Sigma, St. Louis, MO) at two sites on the abdomen. Eight days after immunization, the mice were rechallenged by injection of 20 µl of 5 mg/ml BSA into one rear foot pad, while the other rear foot pad received a comparable volume of phosphate buffered saline (PBS). Measurements of foot pad swelling were taken at 24, 48 and 72 h after challenge by use of micrometer (Mitutoyo, Tokyo, Japan). The magnitude of the DTH response was determined as the differences in foot pad thickness between the antigen and PBS injected foot pads [28].

Effect of oral administration of *Pleurotus* fruiting bodies cold water-extract (CW-E) to malnourished Balb/c mice

Female Balb/c mice, weighing 20 g, were housed individually at 23 °C with a 12-hour/12-hour light/dark cycle. Thirty mice that were starved for 3 days and had free access to salted water were studied. After this time, blood was collected from the orbital vein of 10 mice and the animals were killed (M group). The others were re-fed *ad libitum* for 8 days with commercial pelleted diet (M-DCgroup) or with the commercial diet and the *Pleurotus* fruiting bodies cold water-extract (CW-E) administered orally at a dose of 100 mg/kg of body weight per day (M/CW-E group). A control group of 10 mice was fed with commercial diet throughout the study.

After the small intestine was collected, the segment correspondent to jejunum was rinsed thoroughly with ice-cold saline solution, opened, and blotted dry. The mucosa was scraped with a glass slide and weighed separately. Jejunal mucosa was homogenized with ice-cold phosphate-buffered saline with a pH of 6.0 (1:3 w/v). Total protein and DNA were quantiûed by the methods of Lowry *et al.* [29] and Burton [30], respectively. Humoral immune response was evaluated through an

immunization protocol with sheep red blood cells (SRBC) as antigen. Three groups, comprised of five mice, were designed: M-DC, M/CW-E and control as described above. After the starvation (day 0) mice were injected intraperitoneally (i.p.) with 0.2 ml of a 25% SRBC saline solution. After 7 days from the first injection, blood samples of 50 µl were drawn from the orbital plexus to measure antibody titresby a haemagglutination (HA) reaction. The reciprocal serum dilution, which just gave agglutination, was considered to be the titre. At this time, mice received the second immunization and on day 14, antibody titres were determined.

Effect of intraperitoneal (i.p.) administration of *Pleurotus* mycelium hot-water extract (HW-E) to cyclophosphamide-treated or whole-body irradiated mice

Cyclophosphamide (CY)-treated mice.Fifteen male mice (20-25 g) were divided into two groups. *Pleurotus* mycelium hot-water extract (HW-E) was administered intraperitoneally (i.p.) at 100 mg/kg for 7 days to ten Balb/c mice and cyclophosphamide (CY) USP 23 for injection, obtained from JSLYP (China), at 100 mg/kg was given i.p. on the fifth day. The control group, comprised of five mice, was injected i.p. with physiological saline. On the eighth day, blood was collected from the orbital vein and animals were then bled to death.

Whole-body irradiated mice

Male mice were randomly allocated into two groups (n= 10) for eventual whole-body irradiation with a ^{60}Co source Theratron teletherapy unit (Siemens, Erlanger, Germany) in the Oncological Hospital "Conrado Benítez" (Santiago de Cuba, Cuba) at a dose rate of 0.43 Gy/min for 20 min (date of exposure to be designated Day 0). For the analyses of effects of the mushroom-derived materials, one group of mice was administered the extract intraperitoneally (i.p.) at a dose of100 mg/kg in a volume of 0.2 ml on days -10 to -6 and -2 to +1 with respect to the irradiation. Mice in the control group (n=10) were injected with saline solution in place of the extract; non-irradiated mice were used as negative controls. All mice were euthanized by cervical dislocation 24 h after the final administration of extract or saline and tissues/bloods isolated for analyses.

In both experiments, the blood specimens were analyzed for white blood cell count. Moreover, femoral bone marrow cells were withdrawn with Hanks' solution and counted with a Neubauer chamber (Germany).The effects of the extract on *in vivo* phagocytic activity was estimated by measuring carbon clearance in peripheral blood (as an index of the phagocytic activity of liver and spleen) (see reference [24] for details).

Statistical analysis

The results were expressed as mean ± standard deviation (SD). One-way analysis of variance and *post hoc* Tukey's tests or Kruskal-Wallis rank test followed by the Student-Newman-Keuls test was applied to determine the significance of differences between treatments. The Student's *t*-test was used to compare the two means in the experiments related to the effects of HW-E administration in cyclophosphamide-treated or whole-body irradiated mice.Differences at $p< 0.05$ were accepted as significant. The software Statgraphics Plus v. 5.1 (Statistical Graphics Corporation, 1994-2001) was used in the analysis.

RESULTS AND DISCUSSION

In the last decade numerous reports have been published on preclinical studies and clinical trials related to the functionality and bioactive properties of edible mushrooms and their nutraceutical derivatives, including the immune modulatory effects. The 77% of the products were obtained from the fruiting bodies, commercially grown or harvested from the wild, 21% come from the mycelium and about 2% of filtered culture media [9].

Dried *Pleurotus* mushroom would become an attractive alternative for the development of functional foods and nutraceutical preparations. The powder evaluated in this work contained in terms of dried weight: carbohydrate (55%), protein (25%), fat (4%), total fibre (7.5%), ash (7.57%) and total phenols (138 mg/100 g) with an overall energy value of 336 kcal/100 g. Differences in biosynthesis patterns of cell molecular components in distinct stages of the vital cycle [31] would explain the dissimilarities in biochemical composition of fruiting bodies and mycelium extracts.

Table 2. Mycochemical profile of *Pleurotus* sp. fruiting bodies and mycelium derived aqueous extracts*.

Metabolites	Assays	Fruiting bodies hot-water extract	Fruiting bodies cold-water extract	Mycelium hot-water extract
Alkaloids	Dragendorff	++	+++	++
	Wagner	+	++	+
Terpenoids	Solkowski	-	-	-
	Lieberman-Burchard	+	-	-
Carbohydrates/Glycosides	Molisch	++	+	++
Reducing Sugar	Fehling	++	+	+
	Benedict	+	+	+
Phenols and tannins	$FeCl_3$	+	+	+
Amino acids	Ninhydrin	++	+	++
Flavonoids	Concentrated H_2SO_4	+	+	+
	Rosemheim	+	+	+

*The mycochemicals contained in the aqueous extracts were estimated qualitatively according to Harbourne (1984). Three replicates were used for each assay. (-) none, (+) present, (++) mild, (+++) marked.

Although in our study polysaccharides appear to be the most important bioactive component in *Pleurotus*-derived preparations with respect to immunomodulation, the presence in varying amounts of different secondary metabolites could lead to a synergy in the immune enhancing activity (Table 2). The result of the mycochemical test shows that both *Pleurotus* fruiting bodies and mycelium extracts contain alkaloids, phenolic compounds like flavonoids and tannins, reducing sugars and amino acids.

Fats and oil were generally absent in the extracts due to the polarity of the solvent used in their preparation. Among the mentioned bioactive components identified in aqueous extracts, phenolic compounds have been studied for their antioxidant properties, and they also play an important role in cancer prevention [32]. Reducing sugars are structural constituents of beta-D-glucans, components of mycetes' cell walls with a well-documented immunity-stimulating effect [33]. On the other hand, amino acids are the structural units of proteins that may be associated to polysaccharides to form immunomodulating complexes, like PSP isolated from the mycelium of *Trametes versicolor* [34]. Moreover, fungal immunomodulatory proteins, purified from medicinal mushrooms comprise a group of novel proteins which possess immunomodulatory properties and have a strong potential of being applied to food or pharmaceutical products for commercial development [35].

Immune system is a very complex homeostatic system consisting of a network of interacting cells, tissues and organs. It allows the organism to exist within itself and maintains a surveillance to recognize components considered non self. Among higher fungi investigated for immunomodulating effects, several mushroom species demonstrate great potential and some of them are already commercially developed [36].

The oral administration of *Pleurotus* fruiting bodies powder to immune competent Balb/c mice independently of animal sex led to a significant increase in total leukocyte counts with respect to controls ($p<0.05$) between the interval considered as normal for this rodent strain ($6\text{-}17x10^9$/L)(Fig. 1). These findings would be related with the stimulation of the production of white blood cells precursors in bone marrow by hematopoietic cytokines as part of the action mechanism of metabolites contained in mushrooms preparations.

Although leukocyte populations (lymphocytes and neutrophils) increased in Balb/c mice treated with *Pleurotus* powder as a diet complement, a differential pattern was showed depending on animal sex. In this context, lymphocyte counts were

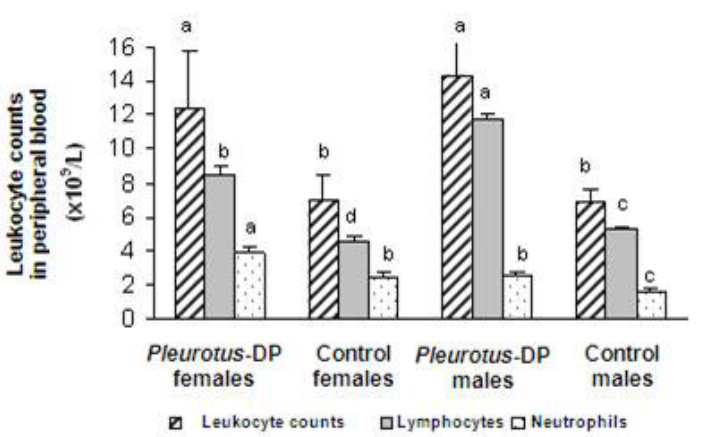

Figure 1. Effect of oral administration of *Pleurotus* fruiting bodies powder on leukocyte counts of immune competent Balb/c mice
All values are given as the arithmetic mean ± standard deviation of 10 mice. Different letters indicate significant differences among the groups (Kruskal-Wallis, Student-Newman-Keuls).

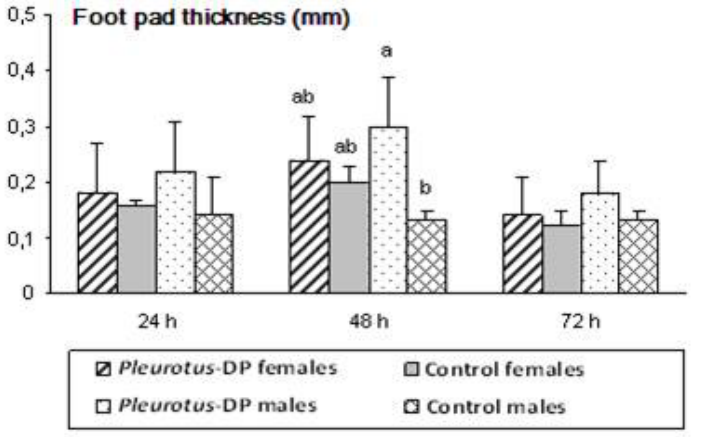

Figure 2. Effect of oral administration of *Pleurotus* fruiting bodies powder on the delayed-type hypersensitivity response (foot pad thickness) of immunocompetentBalb/c mice
All values are given as the arithmetic mean ± standard deviation of 5 mice. Different letters indicate significant differences among the groups (Kruskal-Wallis, Student-Newman-Keuls, $p<0.05$).

higher in males receiving the mushroom preparation while neutrophils exhibited superior values in females ($p<0.05$). The magnitude of delayed-type hypersensitivity reaction (DTH) in male mice, particularly at 48 h after antigen rechallenge ($p<0.05$) (Fig. 2), may reflect the induction of $CD4^+$ Th1 cells and the activation of macrophages by cytokines: tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) [30].

Because most chronic disease states are associated with fasting, which contributes to the establishment of malnutrition,we investigated the effect of *Pleurotus* fruiting bodies cold water-extract (CW-E)on the immunonutritional recovery of malnourished mice.The intestinal tract is an important interface between the organism and the environment. In protein-energy malnutrition, the adaptive responses and defense mechanisms of the gut mucosa are altered [37]. After 72 h of food

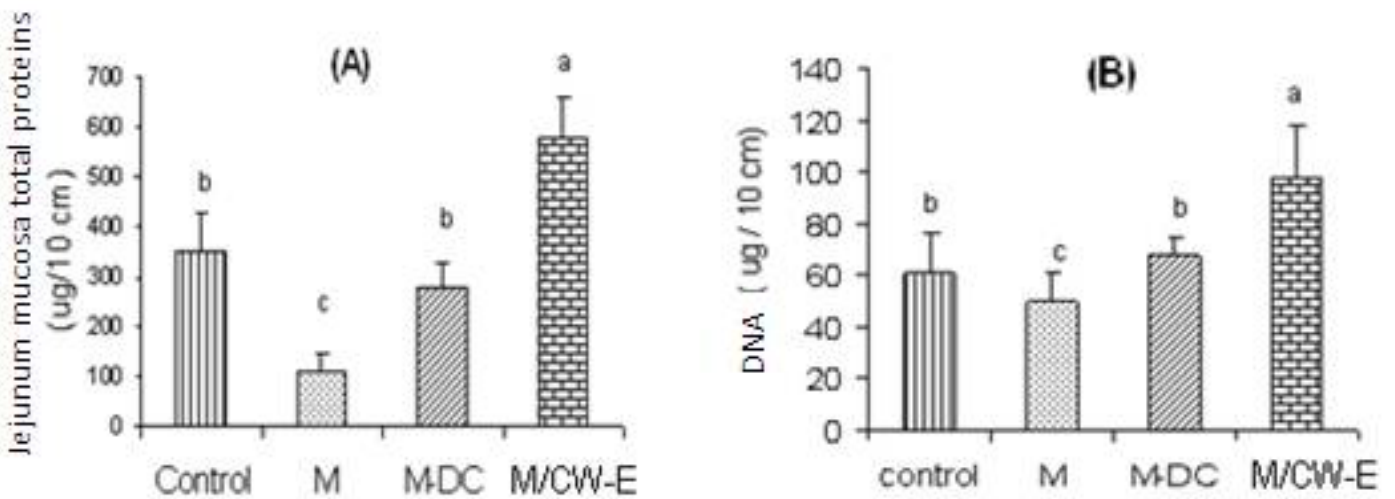

Figure 3. Effect of starvation and refeeding with commercial diet supplemented (M/CW-E) or not (M-DC) with *Pleurotus* fruiting bodies cold water-extract on protein and DNA contents in the jejunum of Balb/c mice.
All values are given as the arithmetic mean ± standard deviation of 10 mice. Different letters indicate signiûcant differences among the groups according to the Tukey test ($p < 0.01$).

deprivation, fasted mice (group M) showed decreases in mucosal DNA and protein contents (Fig. 3). Recovered M-DC mice exhibited a trend toward increased DNA and protein contents, but CW-E refed animals showed values in both gut mucosal protein and DNA higher than control mice ($p<0.05$). The increased DNA content in mice supplemented with CW-E might be associated with the stimulation of protein synthesis, cell division and turnover of enterocytes.

Another effect of CW-E on the immune system was a potentiation of the humoral response, which was determined by measuring antibody titres to SRBC, a T-dependent antigen (Fig. 4). The secondary response (day 14) in CW-E group evoked antibody titres higher than M-DC and controls ($p<0.05$). These findings suggested the stimulation of the functional abilities of Th (T-helper cells) and/or expanded pools of memory B cells by CW-E.

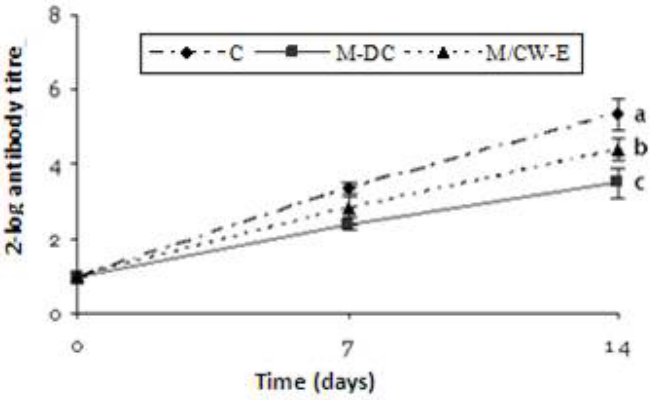

Figure 4. Effect of starvation and refeeding with commercial diet supplemented (M/CW-E) or not supplemented (M-DC) with *Pleurotus* fruiting bodies cold water-extract on humoral immune response against a T-dependent antigen (sheep red blood cells, SRBC).
All values are given as the arithmetic mean ± standard deviation of 5 mice. Different letters indicate signiûcant differences among the groups in Tukey test, $p < 0.05$.

These findings suggest that oral administration of edible mushrooms derived products would stimulate the immune system after their absorption in the gastrointestinal tract and the activation of gut-associated lymphoid tissues, thus integrating different elements of the immune function.An enhancement in Th1 response through intestinal epithelial cells and the suppression of ovalbumin (OVA)-sensitized allergy in mice was reported by Bouike *et al.*[38] as result of the oral treatment with extract of *Agaricus blazei* Murill.

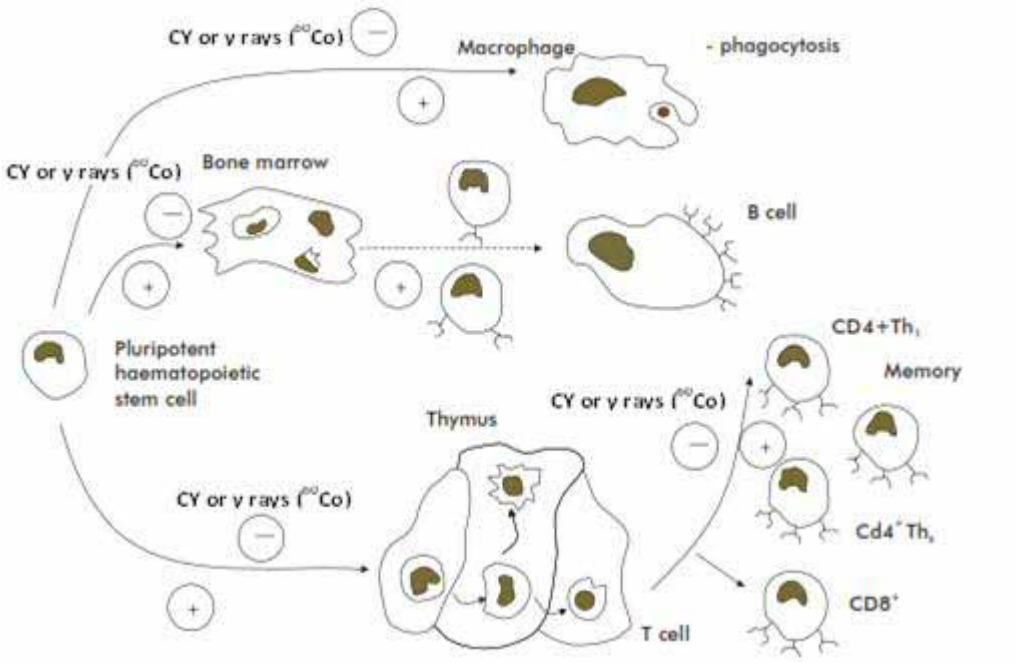

Figure 5. Alterations in the immunological response during cyclophosphamide (CY) or whole-body irradiation (^{60}Co) treatments and the immunoenhancing effects of a mycelium hot-water extract from *Pleurotus* sp. (HW-E) in Balb/c mice. The sign (–) indicates an inhibitory effect of the suppressive treatments and the sign (+) the immunomodulating action of HW-E in immunodeficient mice

The chemotherapy and radiotherapy in cancer treatment contribute to further depression of the immune system. Use of immunomodulating therapeutic agents can solve these problems and efforts to find new immunomodulators are on-going [39]. For that reason, we studied the effects of intraperitoneal administration of *Pleurotus* mycelium hot-water extract (HW-E) to cyclophosphamide-treated or whole-body irradiated mice (Fig. 5).

Cyclophosphamide is probably the most common antineoplastic used in cancer chemotherapy; however, cyclophosphamide shows potent immunosuppressing properties [24]. As expected, cyclophosphamide severely impaired the mice hematopoietic tissue, but *Pleurotus* HW-E was found to have an active protective effect. HW-E increased bone marrow cellularity ($4.1x10^6$ *vs.* $1.5x10^6$ per femur in saline control group, $p< 0.05$), the white blood cell counts ($7.6x10^9$ *vs.* $4.8x10^9$cells/L, $p< 0.05$) and enhanced the monocyte-macrophage system as judged by the shorter rate of carbon clearance (4.23 *vs.* 6.18 min, $p< 0.05$). The stimulant effect on hemopoiesis and cell immune response exerted by a *Pleurotus* fruiting bodies powder administered in a prophylactic schedule to cyclophosphamide treated mice was reported by Morris *et al.*[40].

On the other hand, the radioprotective effect exerted by mycelium HW-E was evident by increases in bone marrow cellularity ($5.1x10^6$ *vs.* $1.1x10^6$/femur in saline-control mice, $p<0.05$), leukocyte counts ($10.5x10^9$ *vs.* $4.5x10^9$/L, $p<0.05$) and the stimulation of macrophage phagocytic activity demonstrated by a faster rate of carbon clearance (1.62 *vs.* 2.01, $p<0.05$). Hence, this extract may be a candidate therapeutic agent with radioprotective activity for hematopoiesis damage, particularly to cells involved in immune function.

Although current knowledge of the role of *Pleurotus*-derived products in the prophylaxis and treatment of diseases are still largely at the empirical level, results obtained in this study demonstrate that not only *Pleurotus* mushrooms but also their mycelia obtained by submerged fermentation may be an interesting renewable resource for developing functional foods, nutraceuticals and new therapeutic agents with immunomodulating activity.

CONCLUSION

Through this immunological window in mushroom biotechnology, we are assisting to a revolution in nutrition and pharmacology. Therefore, attempts to "domesticate" the immune system for the benefit of man, in addition to specific vaccines and antibodies, would find in mushrooms new and unlimited possibilities of exogenous molecules. Our findings provide the basis for submitting the first Cuban dietetic supplement designed from mushroom material "NUTRISETAS®" to the national regulatory agency. Two important challenges for Cuban researchers involved in mushroom science are: (i) the evaluation of both nutritional and pharmacological potentialities of wild mushroom strains and (ii) the developing of new therapies and/or clinical assays based on mushrooms derived preparations alone or combined with traditional therapies. To accomplish these goals in present and future investigations we have to keep in mind the optimization of technologies/ production techniques as well as total quality assurance.

ACKNOWLEDGEMENTS

This work was supported by the University of Oriente under the Institutional project 9615; the FSP Coopération Scientifique Franco-Cubaine, project "Biotechnologie de *Pleurotus* sp. à Cuba et diversification de sa Culture pour des Applications Environementales et Nutraceutique"; and the IRD under the Programme Jeunes Equipes AIRD (JEAI) "Diversification de la Technologie de Culture de *Pleurotus* sp à Cuba et sa Diffusion dans la zone Caraïbe Cari SETA" This work has been also supported by the Belgian Development Cooperation through VLIR-UOS (Flemish Interuniversity Council).

REFERENCES

[1] Chang S and Wasser SP. (2012). The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *Int. J. Med. Mush.* 14: 95-134.

[2] Bloom DE *et al.* (2011). The global economic burden of non-communicable diseases. In: *World Economic Forum*. Available from: http://www.weforum.org/reports/global-economic-burden-non-communicable-diseases.

[3] Cuban Ministry of Public Health. (2014). *Annual Statistical Health 2013.*Direction of Medical Registers and Health Statistics, p. 38, ISSN 1561-4425.

[4] Oz M. (2011). The Oz diet: no more myths, no more fads, what you should eat and why. *Time* Sept 12: 30-35.

[5] Pereira E *et al.* (2012). Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. *Food Chem.* 130: 394-403.

[6] Roupas P *et al.* (2012).The role of edible mushrooms in health: Evaluation of the evidence. *J. Funct. Foods* 4: 687-709.

[7] Zaidman B *et al.* (2005). Medicinal mushrooms modulators of molecular targets as cancer therapeutics. *Appl. Microbiol. Biotechnol.* 67: 453-468.

[8] Ulziijargal E and Mau JL. (2011). Nutrient compositions of culinary-medicinal mushroom fruiting bodies and mycelia. *Int. J. Med. Mush.* 13: 343-349.

[9] Wasser SP. (2010) Medicinal mushroom science: history, current status, future trends, and unsolved problems. *Int. J. Med. Mush.* 12:1-16.

[10] Chang S and Buswell J. (1996). Mushroom Nutriceuticals. *World J. Microb.Biotech.* 12: 473-476.

[11] Wasser SP. (2004). Dietary supplements from culinary-medicinal mushrooms: A variety of regulations and safety concerns for the 21th Century. *Int. J. Med. Mush.* 6: 231-248.

[12] Petrova R *et al.* (2005). Potential role of medicinal mushrooms in breast cancer treatment: current knowledge and future perspectives. *Int. J. Med. Mush.* 7:141-155.

[13] Chang ST. (1999). Global impact of edible and medicinal mush-rooms on human welfare in the 21st century: nongreen revolution. *Int. J. Med. Mush.* 1:1-7.

[14] Wasser SP and Weis AL. (1999). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (review). *Int. J. Med. Mush.*1: 31-62.

[15] Hawksworth DL. (2001). Mushrooms: the extent of the unex-plored potential. *Int. J. Med. Mush.* 3:333-337.

[16] Wasser SP. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotechnol.* 60: 258-274.

[17] Cabarroi M *et al.* (2008). Hongos del Jardín Botánico Nacional de Cuba. I. *Basidiomycota.Revista del Jardín Botánico Nacional* 29: 161-169.

[18] Klibansky MM *et al.* (1993). Production of *Pleurotus ostreatus* mushrooms on sugar cane agrowastes. *Acta Biotechnol.*13: 71-78.

[19] Bermúdez RC *et al.* (2001). Cultivation of *Pleurotus* on agricultural subtrates in Cuba. *Micol. Apl. Int.* 13:25-29.

[20] Martínez-Carrera D and López-Martínez de Alva L. (2010) Historia del cultivo comercial de hongos comestibles en México II: éxitos y fracasos durante el período 1991-2009. In: *Hacia un Desarrollo Sostenible del Sistema de Producción-Consumo de los Hongos Comestibles y Medicinales en Latinoamérica: Avances y Perspectivas en el Siglo XXI.* Martínez-Carrera D. *et al.* Eds. pp. 513-551.

[21] Bermúdez RC *et al.* (2003). Influencia de la luz en la calidad proteica de *Pleurotus ostreatus*var. *florida*. *Rev. Cubana Invest. Bioméd.* 22: 226-231.

[22] Gunde-Cimerman N. (1999). Medicinal value of the genus *Pleurotus* (Fr.) P. Karst.(Agaricaless.I., Basidiomycetes). *Intl. J. Med. Mush.* 1: 69-80.

[23] Gregori A *et al.* (2007). Cultivation techniques and medicinal properties of *Pleurotus* spp. *Food Technol. Biotechnol.* 45: 238-249.

[24] Morris HJ *et al.* (2003). Immunomodulating effects of the hot water extract from *Pleurotus ostreatus* mycelium on cyclophosphamide treated mice. *Micol.Apl. Int.* 15: 7-13.

[25] Patel Y *et al.* (2012). Medicinal properties of *Pleurotus* species (Oyster Mushroom): a review. *World J. Fungal Plant Biol.* 3: 1-12.

[26] Llauradó G *et al.* (2013). Phytochemical screening and effects on cell-mediated immune response of *Pleurotus* fruiting bodies powder. *Food Agric. Immunol.* 24: 295-304.

[27] Harbourne HB. (1984). Phytochemical methods: a guide to modern technique of plant analysis. 2nd ed. London: Chapman and Hall.

[28] Kim YS *et al.* (1998). Targeting the IL-15 receptor with an antagonist IL-15 mutant/$F_{cã}$ 2a protein blocks delayed-type hypersensitivity. *J. Immunol.* 160: 5742-5748.

[29] Lowry H *et al.* (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

[30] Burton K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.* 62: 315-323.

[31] He X and Seleen J. (2004). Chemical analysis as a quality control method for medicinal mushroom and fungi extracts. *Int. J. Med. Mush.* 6: 253-261.

[32] Dubost NJ *et al.* (2007). Quantification of polyphenols and ergothioneine in cultivated mushroom and correlation to total antioxidant capacity. *Food Chem.* 105: 727-735.

[33] Rop O *et al.* (2009). Beta-glucans in higher fungi and their health effects. *Nutr.Rev.* 67: 624-631.

[34] Hobbs C. (2004). Medicinal value of Turkey tail fungus *Trametesversicolor* (L.: Fr) pilát (Aphyllophoromycetideae). a literature review. *Int. J. Med.Mush.* 6: 195-218.

[35] Ou CC *et al.* (2009). Stability of fungalimmunomodulatory protein, FIP-gts and FIP-fve, in IFN-ã production. *Food Agric. Immunol.* 20: 319-332.

[36] Poucheret P *et al.* (2006) Biological and pharmacological activity of higher fungi: 20-year retrospective analysis. *Cryptogamie, Mycologie* 27(4):311-333.

[37] Cummings JH *et al.* (2004). PASSCLAIM-gut health and immunity. *Eur. J. Nutr.* 43 S2: II 118- 173.

[38] Bouike G *et al.* (2011).Oral treatment with extract of *Agaricus blazei* Murill enhanced Th1 response through intestinal epithelial cells and suppressed OVA-sensitized allergy in mice. *Evid-Based Compl. Altern.Med.* Article ID 532180, 11 p.

[39] Zhuang SR. (2009). Effect of citronellol and the Chinese medical herb complex on cellular immunity of cancer patients receiving chemotherapy/radiotherapy. *Phytother. Res*. 23: 785-790.

[40] Morris HJ *et al.* (2011) Immunomodulating properties of *Pleurotus* sp. fruiting bodies powder on cyclophosphamide treated mice. In: Mush. Biol. Mush. Prod. Savoie J.M. *et al.*Eds. 7, 329-338.